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Patentanmeldung Nr. Patent application No. Demande de brevet n°

94203205.3

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Den Haag, den
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31/10/95



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**Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation**

Anmeldung Nr.:
Application no.: 94203205.3
Demande n°:

Anmeldetag:
Date of filing: 03/11/94
Date de dépôt:

Anmelder:
Applicant(s):
Demandeur(s):
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Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:

Use of erythropoietin in the treatment of rheumatoid arthritis

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat:
State:
Pays:

Tag:
Date:
Date:

Aktenzeichen:
File no.
Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:

A61K38/18

Bemerkungen:
Remarks:
Remarques:

13

Title: Use of erythropoietin in the treatment of rheumatoid arthritis.

The invention relates to certain novel uses of the known protein erythropoietin (EPO), or substances having such activity as disclosed herein.

Erythropoietin is a humoral regulator of erythropoiesis, which stimulates the production of erythrocytes. In normal conditions it is produced in sufficient quantities in the kidneys and the liver.

In case of hypoxic shocks (such as massive blood loss) erythropoietin production needs to be increased, which means that it has to be synthesised de novo. In disease-free conditions, erythropoietin levels in circulation are extremely low.

Certain diseases or side-effects of treatments of certain diseases lead to a chronic anaemia which overcharges the capacity of erythropoietin production, or otherwise cannot be met by the body's own erythropoietin resources. These diseases include chronic insufficiency of the kidneys, anaemias associated with malignancies, neonate anaemia, chronic anaemia associated with rheumatoid arthritis (ACD), anaemia after bone marrow transplantation, aplastic anaemia, myeloplastic syndrome and various haemoglobin related diseases. Also anaemic side effects have been shown to occur in various chemotherapies and AZT-therapy. In these cases it may be helpful to administer EPO to increase erythrocyte production.

Human EPO is available as a recombinant protein, which ensures that sufficient quantities can be produced in a very pure form.

Several studies with recombinant human erythropoietin (r-hu-Epo) have been carried out, mainly in patients who underwent renal dialysis for chronic renal failure, in which diminished production of Epo and severe anaemia requiring regular bloodtransfusions occurs. A correction of anaemia by r-hu-Epo was shown in these cases with minimal side-effects

(16,17,18). In AIDS-patients treated with Zidovudine, causing bone marrow suppression, administration of 100 U r-hu-Epo/kg thrice weekly intravenously, significantly decreased transfusion requirements (19).

5 The invention provides a novel use of erythropoietin which is not directly related to its erythrocyte stimulating properties.

This use is specifically clear in rheumatoid arthritis, which therefore is more specifically described as explanatory
10 example for the invention.

Rheumatoid arthritis is an inflammatory disease of synovial membranes, usually expressing itself in a symmetrical polyarthrititis. During the course of their disease 70% of rheumatoid arthritis (RA) patients develop some kind of
15 anaemia (1), which may be due to iron deficiency (2,3), vitamin B12 deficiency or folic acid deficiency (4,5), haemolysis or adverse reactions to anti-rheumatic drugs (6,7). In addition active RA is frequently (in nearly 50%) accompanied by anaemia of chronic disease (ACD) (8).

20 Factors involved in the pathogenesis of ACD are ineffective erythropoiesis (9), interleukin-1 (10), tumour necrosis factor α (TNF- α) (11), decreased erythropoietin synthesis (5,12,13) and/or a decreased response to erythropoietin by the bone marrow (14,15).

25 So far only a few studies with r-hu-Epo have been carried out in RA patients. A haemoglobin (Hb) rise was shown in two anaemic RA patients treated with r-hu-Epo, 125-250 IU/kg thrice weekly, a significant haematocrit rise was recorded (20).

30 We have treated ten RA patients who suffered from ACD with recombinant human EPO.

In all RA patients a rise in haemoglobin was observed. Despite a wide range of values, the increase in haemoglobin became significant after the second week of treatment with
35 recombinant human EPO.

Besides this expected result of EPO treatment a different unexpected benefit was obtained by the treatment.

The invention thus provides the use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of chronic inflammations, especially those related to (auto-)immune diseases, in particular RA. In RA we found an overall improvement in the clinical parameters for scoring disease activity. Most impressive are the results on clinical variables such as painscore and morning stiffness as disclosed below. A significant decrease in the number of tender joints was already observed after two weeks of treatment. The changes in other clinical parameters did not reach statistical significance due to the wide range of values and the small number of patients in the study. However, when the parameters were expressed as percentages of their baseline value, significant improvements were observed.

In addition to this effect on clinical variables a further positive effect was seen in the area of an overall sense of well-being of the treated patients.

According to the invention any erythropoietin which has the ameliorating effect on chronic inflammations can be used. Preferably this erythropoietin is not immunogenic so that it can be administered repeatedly. This will usually lead to the use of human erythropoietin of any origin, although recombinant erythropoietin seems the product of choice because of its purity and constant quality. On the other hand it may very well be possible to use non-human truncated forms of mammalian erythropoietin as long as they have the activity and are not immunogenic upon normal administration to patients. Selected mutants (longer acting, more stable), fragments or derivatives of erythropoietin may also be used as long as they fulfil both criteria.

It is worthwhile to note that patients not having a kind of anaemia can thus be treated with EPO. However, caution has to be taken that Hb-levels do not rise to detrimental levels. Ways of lowering the Hb-levels are well-known in the art.

One of the mechanisms through which EPO may ameliorate the disease symptoms in RA (or other chronical inflammations)

is that it mobilises iron towards haemoglobin production. Iron (free and/or bound in ferritin) deposits are known to occur in the synovia of RA-affected patients. Synovial fluid iron levels correlate with RA activity and therefore it is thought
 5 that iron is involved in the initiation or maintenance of RA synovitis through mediating tissue damage. The role of iron in the pathogenesis of RA may be related to the fact that iron stimulates the production of hydroxyl radicals, which are very potent agents in the destruction of cartilage, membranes and
 10 proteins. A thorough discussion of the role and the mechanisms of iron in the inflamed joint can be found in Vreugdenhil et al. (23). In said study it is suggested to administer iron chelators to RA patients. EPO does not chelate iron. However, EPO does mobilise iron to be incorporated into haemoglobin
 15 through serum transferrin. Thus EPO may reduce the levels of iron in the synovial fluids.

Another possible mechanism which may be responsible for the unexpected beneficial effect of EPO in (especially) RA, may be found in its influence on the T_{H1}/T_{H2} balance.

20 One of the key functional parameters determining the outcome of immune responses, for example infectious agents, is the nature of the cytokines produced locally by immune cells. At this moment evidence is obtained that T-cells can be classified into T_{H1} and T_{H2} cells; both characterized by a
 25 different cytokine secretion profile. T_{H1} cells secrete IL-2 and TNF- γ upon activation but not IL-4 or IL-5, and T_{H2} cells produce IL-4 and IL-5 but not IL-2 or TNF- γ . The differential cytokine profile of these CD4+T cells correlates with different effector functions exerted by these cells: T_{H1} cells
 30 mediate delayed type hypersensitivity (DTH) responses and T_{H2} provide superior help for antibody productions by B cells. There is also some support for the notion that T_{H1} and T_{H2} cells are progeny of T_{H0} cells which can produce IL-2, TNF- γ , IL-4 and IL-5 simultaneously. T_{H1} like cytokine secretion
 35 profile. In different animal studies and observations in human diseases, like leprosy, evidence is obtained that the balance between T_{H1} and T_{H2} response determined the outcome of for

example an infectious disease and disease manifestations. At this moment a selective activation of Th₁-like T cells is proposed as a hallmark of the aethiopathogenesis of rheumatoid arthritis. Evidence for this hypothesis is formed by the fact that on histopathological examination of the synovial tissue, a DTH like of inflammatory reaction is observed which is characteristic for a Th₁ response.

Some observations in our RA patients treated with r-hu-EPO showed a rise in serum IgE levels; which is consistent with the concept that EPO can give support for a Th₂-like response. In other ways influencing the Th₁-Th₂ balance in a more Th₂ cytokine secretion profile. Indirect evidence for this hypothesis is formed by the fact that 2 out of 3 monoclonals raised against EPO are of the IgE class (IgE synthesis is regulated by IL-4).

When EPO is administered to new-born rats a reduced neutrophil production is observed. This reduced neutrophil production may be partly responsible for the ameliorating effect observed in our patients in that neutrophils play a key role in inflammatory reactions.

It has also been observed that EPO can in some ways counteract the activity of TNF- α . TNF- α is an important pro-inflammatory cytokine.

It may also be the case that EPO diverts the multipotent progenitor blood cells to the production of erythrocytes instead of granulocytes.

EXPERIMENTAL

Patients:

This study focused on the effects of r-hu-Epo on RA disease activity parameters. It is a part of a project studying the pathogenesis of ACD and possible therapeutic strategies. The effect of r-hu-Epo on the anaemia and iron metabolism is reported in more detail (21).

Ten patients with RA (22) were studied, fulfilling the criteria for ACD as proposed by Carwright (8). ACD was

confirmed by measuring stainable iron in a bone marrow preparation. Patients treated previously with iron, vitamin B12, folic acid and cytotoxic drugs were excluded. Other causes of anaemia were also excluded such as the presence of
5 haematuria, positive occult bloodtest in stool, decreased creatinine clearance, haemolysis and low vitamin B12 or folic acid.

The demographic features of the studied patients are summarized in table I. All patients used a variety of non
10 steroidal anti-inflammatory drugs.

Treatment:

Recombinant human Erythropoietin (r-hu-Epo, Boehringer, Mannheim, Germany), was administered three times a week at a dose of 240 units/kg subcutaneously at the right upper leg for
15 6 weeks.

Clinical and laboratory monitoring:

Detailed clinical and laboratory evaluation was performed at entry and weekly by the same physician, till the end of the study (6 weeks), then at 9 and 12 weeks after onset of the
20 study. Routine laboratory procedures were used for assessment of haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpus haemoglobin (MCH) and reticulocytes count. Serum iron was measured spectrophotometrically (Instruchemie, Hilversum, the Netherlands). Transferrin and CRP was assessed
25 with a nephelometer (Ablon Medical Systems, Leusden, the Netherlands) and serum ferritin by solid phase enzyme immune assay (Ferrizyme, Abbott Labs, Chicago, USA). The erythrocyte sedimentation rate (ESR) was measured by the Westergren
30 method. The Ritchie index, grip strength, number of swollen joints, morning stiffness and a subjective pain score (visual analogue scale, 0-10 points) were assessed as well. Liver and kidney function tests were performed to monitor possible side effects.

Data evaluation:

For evaluation all clinical data were stored and analyzed on a Wang personal computer using the Lotus 1-2-3 program. Statistical evaluation of the results was by Fishers' exact
5 test for group differences. P values of 0.05 or less were considered significant.

RESULTS

Effect of r-hu-Epo on the anemia of chronic disease (ACD).

In all RA patents a rise in haemoglobin was observed
10 (table II). Despite of the wide range of values, the increase in haemoglobin became significant after the second week of treatment compared to baseline values. When treatment was stopped haemoglobin stayed significant higher compared to the baseline value, but dropped in the 12th week.

15 Iron deficiency developed as shown by the fact that five patients were characterized by ferritin levels lower than 40 µg/ml.

Effect of r-hu-Epo on disease activity parameters.

Laboratory parameters: ESR and CRP.

20 A decrease in ESR was found in all patients (table III), which started at the third week of treatment and remained so until the end of the study. As illustrated the decrease in eight patients was more than 20% of their baseline value; which was highly significant. The same holds true for the CRP
25 values, but due to the wide range in the absolute values and small number of investigated patients, no significance could be calculated. However, expressing the values as a percentage of the baseline value, also in this way after the third week of treatment, a significant decrease in the CRP levels was
30 observed.

Subjective clinical scores: painscore (PS) and morningstiffness (MS).

Both parameters (PS and MS) showed during the follow-up a tendency to decrease (table IV). Caused by the variability in

absolute values and small number of patients a significance could not be calculated. When the values were expressed in a percentage of the baseline value, the PS decreased significantly after the third week of treatment and the MS after the sixth week.

Objective disease activity scores: gripstrength (GS), Ritchie Index (RI) and number of swollen joints (SJ).

All parameters as shown in table V showed a continuous tendency towards improvement which lasted during, and also after, the treatment period. In the absolute changes in number of tender joints a significant decrease could be calculated from the third week of treatment. Also a continuous decrease in the number of swollen joints was observed from T3 on and at T9 nine out of ten patients had less swollen joints, which was highly significant.

Caused by the variation of the individual values of the GS, it was impossible to calculate a significance. However, when the values were expressed as a percentage of their baseline values after three weeks of treatment, a significant increase in GS was noted. It should be mentioned that the GS remained stable in three patients during our investigation.

TABLE I

Demographic features of ten patients characterized on having anaemia of chronic disease (ACD) and rheumatoid arthritis (RA)

Female/Male		9/1
Mean age (years)		68 \pm 6,5
Treatment:		
Prednisolone	(2 patients)	5 mg 1.5-2.5 g/day
Sulphasalazine	(3 patients) (range)	200 mg/day 50 mg/in 2 weeks
Plaquenil	(1 patient)	500-750 mg/day
Auromyose	(1 patient)	
D-Penicillamine	(2 patients) (range)	

- 5 All patients were treated for more than 2 months with the mentioned disease modifying anti-rheumatic drugs.

TABLE II

Effect of recombinant human erythropoietin (r-hu-Epo) therapy on haemoglobin and ferritin levels at the defined time periods after onset therapy in ten patients with rheumatoid arthritis (RA)

5

Variable	Base-line	Values during the 6 weeks therapy and after 3 and 6 weeks of treatment.							
	TO*	T1	T2	T3	T4	T5	T6	T9	T12
Hemo-globin	5.9	6.1	6.5**	6.8	7.0	7.2	7.2	7.2	6.6
mmol/l	0.4	0.5	0.6	0.7	0.9	1.0	1.0	1.1	0.9
± sd									
Ferritin material	216		143**				80	49	61
µg/ml	140-318		44-301				14-157	19-82	52-84
Range									

* Refers to treatment weeknumber.

** Marks the treatment period when the differences between baseline became significant.

10

TABLE III

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels at the defined time periods after onset therapy in ten patients with rheumatoid arthritis (RA)

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
ESR (mmH)				
mean	82	61**	53**	56**
ranges	42-137	18-112	7-98	7-111
ESR (%)				
mean	100	63	59	64
ranges	-	32-107	16-108	16-144
Number of patients with a change > 20% baseline value	-	8**	7**	8**
CRP (mg/l)				
mean	51	45	43	44
ranges	10-105	4-113	3-122	1-144
CRP (%)				
mean	100	85	85	81
ranges	-	17-155	8-204	5-181
Number of patients with a change > 20% baseline value	-	5**	6**	6**

- * Refers to treatment weeknumber.
 10 ** Marks the treatment period when the differences compared to baseline values became significant.
 P > 0.05, Fishers's exact test.

TABLE IV

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the overall pain score (PS) and morning stiffness duration (MS) at the defined time periods after onset of treatment in ten patients with rheumatoid arthritis (RA).

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
PS				
mean	3.9	3.0	2.7	2.8
ranges	2.7	1-5	1-5	1-5
PS (%)				
mean	100	82	70	73
ranges	-	50-150	33-150	33-100
Number of patients with a change > 20% baseline value	-	7**	8**	6**
MS (min)				
mean	45	37	35	36
ranges	10-120	10-120	10-120	10-120
MS (%)				
mean	100	88	78	85
ranges	-	50-150	50-150	50-150
Number of patients with a change > 20% baseline value	-	3	5**	5**

* Refers to treatment weeknumber.

** Marks the treatment period when the differences compared to baseline values became significant.
P > 0.05, Fishers's exact test.

TABLE V

Effect of recombinant human erythropoietin (r-hu-Epo) treatment on the Ritchie index (RI), number of swollen joints (SJ) and grip strenght (GS) at the defined time periods after onset treatment in ten patients with rheumatoid arthritis (RA).

Variable	Baseline	Values during 6 and 3 weeks after the end of treatment period.		
		T3*	T6	T9
RI mean ranges	13 3-38	10.2 1-22	7.7** 1-14	6** 2-13
RI (%) mean ranges	100 -	66 25-100	62 33-111	56 22-95
Number of patients with a change > 20% baseline value	-	8**	7**	9**
SJ mean ranges	8 6-5	6 3-11	4.5 2-8	4.5 1-9
SJ (%) mean ranges	100 -	72 42-100	61 37-100	51 20-100
Number of patients with a change > 20% baseline value	-	8*	7*	9*
ESR (mmH) mean ranges	72 15-190	87 20-220	91 20-220	90 15-220
ESR (%) mean ranges	100 -	112 90-133	118 90-166	118 90-166
Number of patients with a change > 20% baseline value	-	4**	4**	5**

* Refers to treatment weeknumber.

10 ** Marks the treatment period when the differences compared to baseline values became significant.
P > 0.05, Fishers's exact test.

CLAIMS

1. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of chronic inflammations.
- 5 2. Use according to claim 1, wherein the inflammation is associated with an immune disease.
3. Use according to claim 2 wherein the immune disease is an auto-immune disease.
- 10 4. Use according to claim 3, wherein the auto-immune disease is rheumatoid arthritis.
- 15 5. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the treatment of symptoms associated with rheumatoid arthritis.
- 20 6. Use according to claim 5, wherein the symptoms treated comprise at least one of the group of morning stiffness, painful and swollen joints, loss of grip strength and pain.
- 25 7. Use of erythropoietin or a substance having erythropoietin-like activity in the preparation of a pharmaceutical for the amelioration of disease activity of rheumatoid arthritis.
- 30 8. Use according to anyone of the afore going claims, wherein the erythropoietin is human erythropoietin.
9. Use according to anyone of the foregoing claims wherein the erythropoietin or the substance having such activity is of recombinant origin.

ABSTRACT

Title: Use of erythropoietin in the treatment of rheumatoid arthritis.

A novel use of the known protein Erythropoietin (EPO) and/or a derivative and/or a fragment thereof is disclosed. EPO is used as a pharmaceutical for the treatment of chronic inflammations. A particularly beneficial result is seen in patients suffering from rheumatoid arthritis (RA). Significant effects are seen in clinical variables such as morning stiffness, swollen joints, and the like.

References

1. Mowat MG: Hematologic abnormalities in rheumatoid arthritis. *Semin Arthr Rheum* 1971; 1:383-390.
2. Hansen TM, Hansen HE, Birgens HS, Hölund B, Lorenzen I: Serum ferritin and the assessment of iron deficiency in rheumatoid arthritis. *Scand J Rheumatol* 1983; 12:353-359.
3. Vreugdenhil G, Baltus CAM, Van Eijk HG, AJG Swaak: Anemia of chronic disease. Diagnostic significance of erythrocyte and serological parameters in iron deficient rheumatoid arthritis patients. *Br J Rheumatol* 1990; 29:105-110.
4. Couchman KG, Bieder L, Wigley RD, Glenday AG: Vitamin B12 absorption and gastric antibodies in rheumatoid arthritis. *NZ Med J* 1968; 153-156.
5. Vreugdenhil G, Wognum AW, Van Eijk HG, AJG Swaak: Anemia in rheumatoid arthritis. The role of iron, vitamin B12 and folic acid deficiency and erythropoietin responsiveness. *Ann Rheum Dis* 1990; 49:93-98.
6. Van de Putte LBA: Pancytopenia related to azathioprine in rheumatoid arthritis. *Ann Rheum Dis* 1988; 47(6):503-505.
7. Dinant HJ, De Maat CEM: Erythropoiesis and mean cell lifespan in normal subjects and patients with the anemia of rheumatoid arthritis. *Br J Hematol* 1979; 39:437-444.
8. Cartwright GE and Lee GR: The anemia of chronic disorders. *Br J Hematol* 1971; 21:147-152.
9. Williams RA, Samson D, Tikerpae J, Crowne H, Gumpel JM: In vitro studies of ineffective erythropoiesis in rheumatoid arthritis. *Ann Rheum Dis* 1982; 41:502-507.
10. Maury CPJ, Andersson LC, Teppo AM, Patanen S, Juronen E: Mechanisms of anemia in rheumatoid arthritis: demonstration of raised interleukin-1 beta concentrations in anemic patients and of interleukin-1 beta mediated suppression of normal erythropoiesis and proliferation of human erythroleukemia cells in vitro. *Ann Rheum Dis* 1988; 47:972-978.

11. Roodman GD: Mechanisms of erythroid suppression in anemia of chronic disease. *Blood Cells* 1987; 13:171-184.
12. Ward HP, Gordon B, Picket JC: Serum levels of erythropoietin in rheumatoid arthritis. *J Lab Clin Med* 1969; 74:93-97.
13. Baer AN, Dessypris N, Goldwasser E, Krantz SB: Blunted erythropoietin response to anemia in rheumatoid arthritis. *Br J Hematol* 1987; 66:559-564.
14. Zucker S, Lysik RM, Di Stefano M: Cancer cell inhibition on erythropoiesis. *J Lab Clin Med* 1980; 99:770-782.
15. Harvey AR, Clarke BJ, Chui DHK, Kean F, Buchanan WW: Anemia associated with rheumatoid disease. Inverse correlation between erythropoiesis and both IgM and rheumatoid factor levels. *Arthr Rheum* 1983; 26:28-34.
16. Esbach JW, Egrie JC, Downing MR: Correction of the anemia of endstage renal disease with recombinant human erythropoietin results of a phase I and II clinical trial. *N Eng J Med* 1987; 316:73-78.
Urabe A, Tokaku F, Mimura N: Therapeutic effect of recombinant human erythropoietin in anemia caused by chronic renal disease. *Exp Hematol* 1987; 15:438-441.
18. Ponticelli C, Casat S: Correction of anemia with recombinant human erythropoietin. *Nephron* 1989; 52:201-208.
19. Fischl M, Galpin JE, Levine JD: Recombinant human erythropoietin for patients with AIDS treated with Zidovudine. *N Eng J Med* 1990; 21:1488-1493.
20. Pincus T, Olsen NJ, Russel IJ: Multicenter study of recombinant human erythropoietin in correction of anemia in rheumatoid arthritis. *Am J Med* 1990; 89:161-168.
21. Vreugdenhil G, Manger B, Nieuwenhuizen C, van Eijk HG, Swaak AJG: Iron stores and serum transferrin receptor levels during recombinant human erythropoietin treatment of anemia in rheumatoid arthritis. *Ann of Hematol* 1992; 65:265-268.

22. Arvett FC, Edworthy S, Bloch DE: The American Rheumatism Association 1987, revised criteria for the classification of rheumatoid arthritis. *Arthr Rheum* 1988; 31:315-324.
23. Synovial Iron Deposition and Rheumatoid Arthritis.
G Vreugdenhil and AJG Swaak in: Handbook on metal-ligand interactions in biological fluids vol. 2 part four, chapter 5 section c. Editor: Guy Berthon: 1993.